REMARKS

Claims 1-8 have been canceled without prejudice. Claim 9 has been amended to add the phrase "and wherein the hepatocytes are capable of maintaining functionality *in vitro* when seeded on the liver basement membrane wherein functionality is selected from the group consisting of albumin production, urea production, and cytochrome P450 activity." Support for amended claim 9 can be found in the specification, for example, on pages 21-23, and throughout the application as filed.

Rejection of Claims 1-16 Under 35 U.S.C. §103(a)-

The Examiner has rejected claims 1-16 under 35 U.S.C. §103 as being obvious over WO 98/25637 and U.S. Patent No. 6,793,939 (the '939 patent). These documents are the PCT publication and an issued U.S. patent based on the PCT application from which the WO 98/25637 publication resulted. The Examiner contends that one would have a reasonable expectation of success in repairing liver tissue based on the disclosure in these documents. The Examiner argues that hepatocytes are mentioned in the cited references and that hepatocyte growth and proliferation would achieve regeneration of liver tissue. Thus, according to the Examiner, the invention is suggested by the cited references.

Although Applicants disagree that the Examiner has established a *prima facie* case of obviousness sufficient to support the Examiner's rejections under 35 U.S.C. §103, the Examiner's rejections under 35 U.S.C. § 103(a) are overcome by unexpected results obtained by Applicants. Applicants have shown that *functional* hepatocytes can be maintained in culture using liver basement membrane as a substrate. As the court concluded in *In re Diamond*, the question of nonobviousness must turn on whether the *prima facie* case of obviousness of the claimed composition is rebutted by a showing of unexpected results. *In re Diamond*, 53 CCPA 1172, 360 F.2d 214, 149 USPQ 562 (1966). *In re Meinhardt*, 55 CCPA

1000, 392 F.2d 273, 157 USPQ 270 (1968).

As indicated by the Examiner, the '637 application and the '939 patent mention that liver basement membranes might be useful to *stimulate proliferation* of undifferentiated or differentiated cells (column 8, lines 55-59 and page 12, lines 3-6, respectively). Each reference also lists examples of differentiated cells, including hepatocytes. However, even if one skilled in the art expected to stimulate the *proliferation* of hepatocytes (*i.e.*, increase their number) by using liver basement membrane compositions as a substrate, there is no expectation that liver basement membranes could be used for ***maintenance of hepatocyte functionality.***

Mere hepatocyte proliferation in culture does not translate into maintenance of hepatocyte functionality. The difficulty in maintaining the functionality of hepatocytes in culture is well-known to those skilled in the art. Therefore, one skilled in the art would not expect to maintain ***functional*** hepatocytes using liver basement membrane as a substrate. Numerous publications provide evidence that those skilled in the art would not expect that hepatocytes cultured on liver basement membrane would maintain hepatocellular function, as was surprisingly found by Applicants. For example, see Yamamoto et al., *Hepatology Research* 35(3):169-77 (2006) (abstract), stating that "Long-term culture of primary hepatocytes from various species is impeded by a decrease in cell viability and a *loss of hepatocytes-specific function.*" (emphasis added); Wang YJ et al., *World J. Gastroenterol.* 10(5):699-702 (2004) stating that "[I]t is *difficult to maintain the physiological function of hepatocytes*, leading to restriction of their extensive uses." (emphasis added); and Campbell LH et al., *In Vitro Cell Dev. Bio. Meeting,* 2007, (abstract A-2000) stating "[Hepatocyte] *specific functions are lost in culture relatively quickly.*" (emphasis added). These references were included as references 1-3 in an Information Disclosure Statement submitted with Applicants' last response.

Specifically, hepatocyte culture on liver basement membrane was found by Applicants to be unexpectedly superior to conventional hepatocyte culture on adsorbed collagen and hepatocytes were found to maintain synthetic and metabolic functions when cultured on liver basement membranes. For example, albumin production, a measure of liver synthetic function, was found to be maintained or elevated in hepatocytes cultured on liver basement membrane, whereas albumin production declined in hepatocytes cultured on adsorbed collagen. (See page 22, lines 19-21 in the instant application). The synthetic and metabolic functions were not only superior to culture on adsorbed collagen, but were also comparable to culture on a double-gel collagen substrate, a substrate with known capacity for maintaining hepatocyte synthetic and metabolic functions. (See page 22, lines 16-18 in the instant application).

Also, urea content, a measure of liver metabolic function, was found to be about the same in hepatocytes cultured on liver basement membrane, on a per cell basis, as that from cells grown on a double-gel substrate. (See page 22, line 32 to page 23, line 1 in the instant application). Additionally, in hepatocytes cultured on liver basement membrane, cytochrome P450 activity, a measure of liver metabolic activity, was at least as high if not greater than that for hepatocytes grown on a double-gel substrate. (See page 23, lines 27-31 in the instant application). These results together show that hepatocytes grown on liver basement membranes exhibit specific liver synthetic and metabolic activity characteristic of ***functional hepatocytes***, results that are unexpected based on the well-known difficulties associated with maintaining hepatocellular-specific functions in cultured hepatocytes.

Accordingly, even if the Examiner has established a *prima facie* case of obviousness, and Applicants contend that the Examiner has not, Applicants have overcome the Examiner's *prima facie* case of obviousness by demonstrating that Applicants' claimed methods and compositions unexpectedly result in a level of hepatocyte functionality that is

difficult to obtain.

In response to our previous arguments regarding unexpected results (i.e., maintenance of hepatocellular-specific functions in hepatocytes cultured on liver basement membrane), the Examiner contends that 1) Applicants are arguing limitations not presently in the claims, and 2) there is insufficient evidence provided to show unexpected results. As to the Examiner's argument that limitations not presently in the claims are argued, Applicants have amended claim 9 to add the phrase "and wherein the hepatocytes are capable of maintaining functionality *in vitro* when seeded on the liver basement membrane wherein functionality is selected from the group consisting of albumin production, urea production, and cytochrome P450 activity." Thus, Applicants are arguing limitations present in the claims although Applicants' understanding is that unexpected results do not have to be claimed to make arguments based on unexpected results.

The Examiner also argues that our previously submitted evidence (Yamamoto et al., Wang YJ et al., and Campbell LH et al.) discuss both 1) the difficulties associated with hepatocyte culture, and 2) culture techniques developed to overcome the difficulties of maintaining hepatocyte function. Thus, the Examiner infers that a skilled artisan would not have believed at the filing date of the application that it was difficult to maintain hepatocellular-specific functions in cultured hepatocytes. Additionally, the Examiner argues that the double gel culture system, used in the instant application as a positive control, was developed before the filing date and shows maintenance of hepatocellular-specific functions. The Examiner further argues that Applicants are growing hepatocytes on their native substrate (i.e., liver basement membrane), and that cells grown on the same substrate found *in vivo* would be expected to exhibit their normal metabolic functionality.

First, the references cited in our previous response do indeed show that hepatocyte function was known to be difficult to maintain in cell culture, and this fact is

conceded by the Examiner. However, the Examiner's argument that the references also teach culture techniques to overcome these difficulties, with the inference that a skilled artisan would not have believed at the filing date of the application that it was difficult to maintain hepatocellular-specific functions in cultured hepatocytes, has no merit. The references cited in the previous response were published in 2004, 2006, and 2007, all after the 2002 priority date of the instant application. Thus, as late as 2007, and certainly at the filing date of the instant application in 2002, hepatocellular-specific functions were known to be difficult to maintain *in vitro*. The fact that techniques were developed after the filing date to grow hepatocytes in culture, or to maintain functionality, is irrelevant.

Because hepatocytes were known to be difficult to grow, as of the filing date of the instant application, there was no expectation that liver basement membrane could be used for ***maintenance of hepatocyte functionality***, as claimed in amended claim 9. Moreover, the fact that one complex method for maintaining hepatocyte functionality *in vitro* (i.e., the double gel substrate method) had been developed before the filing date of the application does not change the fact that hepatocellular-specific functions were known to be difficult to maintain *in vitro* at the filing date. Also, growth on the native substrate (i.e., liver basement membrane) would not necessarily be expected to maintain hepatocellular-specific functions *in vitro*. If liver basement membrane was expected to maintain hepatocellular-specific functions *in vitro*, scientists would not have invested the time and effort to develop other complex methods of maintaining hepatocyte functionality *in vitro*, such as the double gel substrate method. Withdrawal of the rejection of claims 9-16 under 35 U.S.C. § 103(a) is respectfully requested.

Double Patenting Rejection

Claims 1-8 rejected for double patenting have been canceled without prejudice so this rejection is moot.

CONCLUSION

The claims are believed to be allowable. Passage of the application to issuance is requested.

Respectfully submitted,

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